

Please replace the following paragraphs in the specification as follows:

At page 3, lines 4 through 14:

P1

The invention also relates to nucleic acid molecules. For example, a nucleic acid molecule can comprise a nucleotide sequence depicted in Figure 9 (SEQ ID NO:10), nucleotides 37 - 150 of Figure 9 (nucleotides 37-150 of SEQ ID NO:10), nucleotides 37 - 186 of Figure 9 (nucleotides 37-186 of SEQ ID NO:10), Figure 10 (SEQ ID NOS:12-14), nucleotides 1421 - 1566 of Figure 10 (nucleotides 1421-1566 of SEQ ID NOS:12-13), nucleotides 1457 - 1566 of Figure 10 (nucleotides 1457-1566 of SEQ ID NOS:12-13), Figure 15 (SEQ ID NOS:21 and 23) or Figure 16 (SEQ ID NO:10). The nucleic acid molecule can also comprise a nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in a row of Figure 1 (SEQ ID NOS:1-8), amino acids 12 - 48 of a row of Figure 1 (amino acids 12-48 of SEQ ID NOS:1-8), the top row of Figure 3C (SEQ ID NO:9), Figure 9 (SEQ ID NO:11), amino acids 12 - 48 of a row of Figure 9 (amino acids 12-48 of SEQ ID NO:11), Figure 10 (SEQ ID NOS:15-17), amino acids 12 - 88 of Figure 10 (amino acids 12-88 of SEQ ID NOS:15-17), Figure 11B (SEQ ID NOS:18-20) or Figure 17 (SEQ ID NO:25). Also included are complementary strands of these sequences, DNA sequences that hybridize to the sequences, RNA sequences transcribed from the sequences, or a fragment or mutation thereof, which encodes a coiled-coil oligomer.

At page 3, line 19 through page 4, line 6:

Also encompassed in the scope of the invention are isolated, purified and/or recombinant peptides and molecules comprising peptides. In one embodiment, a polypeptide comprises an amino acid sequence encoded by an HDAg nucleic acid molecule. The molecules can comprise a polypeptide having an amino acid sequence selected from the group consisting of an amino acid sequence depicted in a row of Figure 1 (SEQ ID NOS:1-8), amino acids 12 - 48 of a row of Figure 1 (amino acids 12-48 of SEQ ID NOS:1-8), amino acids 12 - 60 of a row of Figure 1

B² (amino acids 12-60 of SEQ ID NOS:1-8), the top row of Figure 3C (SEQ ID NO:9), Figure 9 (SEQ ID NO:11), amino acids 12 - 48 of Figure 9 (amino acids 12-48 of SEQ ID NO:11), amino acids 12 - 60 of Figure 9 (amino acids 12-60 of SEQ ID NO:11), Figure 10 (SEQ ID NOS:15-17), Figure 11B (SEQ ID NOS:18-20) and Figure 17 (SEQ ID NO:25), or a fragment or derivative thereof which forms a coiled-coil oligomer. The peptide can be a derivative peptide wherein a serine residue is substituted with cysteine. The molecules can comprise a polypeptide comprising an amino acid sequence of amino acids 12 - 88 of HDAG, or a fragment or derivative thereof which forms a coiled-coil oligomer and nuclear localization signal. The polypeptides can be encoded by fusion genes comprising HDAG. It is possible that the molecule can be larger than the 12-48 or 12-60 or 12-88 amino acids, for example. It may be desirable to make a 12-65 or 10-93 peptide, for example.

At page 6, lines 7 through 12:

B³ Figure 1 depicts sequence alignment of 11 serotypes of hepatitis delta antigen (HDAG) between amino acids 12 to 60 (SEQ ID NOS:1-8). Asterisks, *, indicate residues which make up the a and d positions in the heptad repeat in the predicted coiled-coil region. Bold pink and purple indicate residues involved in the hydrophobic interactions in the dimer between the two termini. The "ds" indicate residues involved in the dimer-dimer interface. A region (pink), B (green), C (purple).

At page 6, lines 18 through 27:

B⁴ Figure 3A depicts C α trace of the peptide δ 12-60(Y). A region pink, B region green, and C region purple. The individual helix takes a sharp bend at proline 49 (Pro49). Figure 3B is a ribbon diagram of the view in Figure 3A rotated 90° along the horizontal axis. The sidechains have been added and the C region of the peptide (residues 50-60(Y)) has been removed for clarity. Sidechains are colored as follows: hydrophobic gray, polar yellow, acidic red and basic blue. Figure 3C (SEQ ID NO:9) is the amino acid sequence of the long helix formed from residues 12 to 48 displayed in the antiparallel orientation of the peptide. The letters above the

B4 amino acid sequence represent the heptad repeat (abcdefg)_n where the a and d residues tend to be hydrophobic. The residues involved in the heptad repeat at the a and d positions are shown in bold.

At page 7, line 25 through page 8, line 3:

B5 Figure 9 (SEQ ID NOS:10 and 11) depicts the sequence of a synthetic gene for optimized expression of HDAG-S in E.coli. The synthetic gene (SEQ ID NO:10) has been modified such that the codon usage which is unusual in the natural gene is assistant with the known preferences for codon usage in E. coli. The underlined sequences correspond to the eight primers used in the first round of PCR. The primers used in the second round are indicated with a dotted underline. The amino acid sequence (SEQ ID NO:11) is shown above the DNA sequence by the one-letter amino acid code. The restriction sites used in cloning are shown in italics.

At page 8, lines 4 through 5:

B6 Figure 10 (SEQ ID NOS:12 -17) depicts the complete sequence of human HDV cDNA (SEQ ID NO:13), and the predicted amino-acid sequence (SEQ ID NO:15) of human HDV delta antigen.

At page 8, lines 6 through 11:

B7 Figure 11 depicts synthetic peptides from the multimer-forming domain of HDAG. (A) Structural organization of HDAG. The lightly stippled region is the multimer-forming domain (amino acid residues 12-60) (SEQ ID NO:1), the solid regions are the RNA-binding domains, and the heavily stippled region is the C-terminal extension of large HDAG. Hydrophobic residues contributing to the heptad repeat are shown in boldface type, (B) Amino acid sequences of three HDAG peptides (SEQ ID NOS:18-20).

At page 9, lines 10 through 11:

B⁸

Figure 15 (SEQ ID NOS:21-24) is a comparison of the wildtype nucleotide sequence of HDAG-S (SEQ ID NO:23) and the sequence of the synthetic HDAG gene for optimized expression in *E. coli* (SEQ ID NO:21).

At page 9, lines 12 through 13:

B⁹

Figure 16 is the nucleotide sequence of the synthetic open reading frame (ORF) for the synthetic HDAG (SEQ ID NO:10).

At page 9, lines 14 through 15:

B¹⁰

Figure 17 is a comparison of the protein amino acid sequence (SEQ ID NO:25) encoded by the wildtype ORF and the synthetic ORF, showing complete (100%) identity.

At page 9, lines 16 through 19:

B¹¹

Figure 18 depicts the nucleotide sequences of the primers used for the two polymerase chain reactions (PCR) to create the synthetic gene. Primer1 - primer8 (SEQ ID NOS:26-33) were used in the first round of PCR and primer9 - primer10 (SEQ ID NOS:34-35) were used in the second round of PCR.

At page 11, lines 3 through 11:

B¹²

An HDAG peptide can, e.g., include all or a portion of the amino acids depicted in Figures 1, 3C, 10, 11B or 17 (SEQ ID NOS:1-8, 9, 15-17, 18-20 and 25). An HDAG peptide can be encoded by an isolated and/or purified or recombinant nucleic acid molecule or a fusion gene (nucleic acid molecule) such as those described herein. In a preferred embodiment, an isolated and purified polypeptide has an amino acid sequence depicted in a row of Figure 1 (SEQ ID

B¹² NOS:1-8), amino acids 12-48 of a row of Figure 1 (amino acids 12-48 of SEQ ID NOS:1-8), amino acids 12-60 of a row of Figure 1 (amino acids 12-60 of SEQ ID NOS:1-8), a row of Figure 3C (SEQ ID NO:9), Figure 9 (SEQ ID NO:11), amino acids 12-48 of Figure 9 (amino acids 12-48 of SEQ ID NO:11), amino acids 12-60 of Figure 9 (amino acids 12-60 of SEQ ID NO:11), Figure 10 (SEQ ID NOS:15-17), Figure 11B (SEQ ID NOS:18-20), Figure 17 (SEQ ID NO:25) or a fragment or derivative thereof, which forms a coiled-coil oligomer.

At page 11, lines 15 through 23:

B¹³ "Homology" is defined herein as sequence identity. Preferably, the protein or polypeptide shares at least about 50 % sequence identity or homology and more preferably at least about 75 % identity or at least about 90% identity with the corresponding sequences of the native protein, for example, with Figure 10 (SEQ ID NOS:15-17). The phrase "substantially the same sequence" is intended to include sequences which bind the viral protein and possess a high percentage of (e.g., at least 90%, preferably at least about 95%) amino acid sequence identity with the native sequence. For example, a derivative, e.g., a mutant or variant can possess substantially the same amino acid sequence as the native protein.

At page 11, lines 24 through page 12, line 16:

B¹⁴ The modifications to the amino acid sequence (substitutions) can be conserved or non-conserved, natural or unnatural amino acids. The residues that function to form or stabilize the coiled-coil domain or binding sites thereof can be substituted, e.g., conservatively, or they can be maintained. Amino acids of the native sequence for substitution, deletion or conservation can be identified, for example, by a sequence alignment between proteins from different serotypes from related species or other related proteins. In one embodiment, the amino acids which are deleted, added or substituted are amino acids which are not "conserved" between serotypes or species, for example, the amino acids so identified in the sequence alignment exemplified in Figure 1 (SEQ ID NOS:1-8). Conserved amino acids may also be substituted. In one embodiment they are substituted conservatively, for example, substituted by structurally similar amino acids. The

B¹⁴

phrase "conservative amino acids substitutions" is intended to mean substitutions of amino acids which possess similar side chains (e.g., hydrophobic, hydrophilic, basic acidic, aromatic, and aliphatic) as is known in the art. See, for example, Hermanson, G.I. Bioconjugate Techniques, Academic Press, Inc. San Diego, CA (1996). Conservative substitutions include amino acid substitutions of one hydrophobic amino acid for another, for example within the following grouping: W, F, A, P, L, M, I, V. Acidic amino acids include E and D; basic amino acids include K, R, and H. Polar amino acids include S, T, N, Q and G and amide residues include Q and N. An example of a suitable derivative or mutant of the HDAG protein is a protein possessing a consensus sequence of the originating species.

At page 12, lines 17 through page 13, line 11:

B¹⁵

In one embodiment, the derivative does not contain substitutes of the residues of Arg13, Leu17, Trp20, Arg24, Trp50 or Leu51. In another embodiment, it contains only conservative substitutions in this region. Hydrophobic residues, for example Ile16, Leu17, Trp20, Trp50 and Leu51 can be maintained, or they can be replaced with other hydrophobic amino acids, for example, those from the group consisting of Trp, Phe, Ala, Pro, Leu, Met, Ile and Val. In another example, the residues Glu31, Lys38, Trp20 and Glu45 are not substituted or are substituted conservatively. In addition, Arg13 and Arg24 can be maintained (not substituted) or substituted conservatively. In another embodiment, the residues of Figure 1 (SEQ ID NOS:1-8) which are involved in hydrophobic interactions are substituted with other hydrophobic residues. In Figure 3A, hydrophobic residues can be substituted for other hydrophobic residues, polar residues can be substituted for other polar residues, acidic residues can be substituted for other acidic residues, and/or basic residues for other basic residues. In one example, the residues labeled in Figure 3A can be maintained (not substituted) or can be replaced with amino acids with similar characteristics. The amino acids at the 'a' and 'd' positions of the heptad repeat (for example, those indicated with an asterisk in Figure 1 (SEQ ID NOS:1-8) or those listed in bold in Figure 3C (SEQ ID NO:9), can be conserved (maintained), or they can be substituted conservatively, e.g., replaced with hydrophobic amino acids. The residues involved with the dimer-dimer interface (e.g., residues marked with a 'd' in Figure 1 (SEQ ID NOS:1-8) or residues labeled in

B¹⁵ Figure 6) can be maintained. The residues indicated in Figure 4 can be maintained. The derivatives, e.g. mutant and wild-type peptides, can crystallize isomorphously. In one preferred embodiment, at least one serine residue, e.g., Ser22, is replaced with a cysteine. In another embodiment, Trp20 is replaced with Ala20.

At page 14, lines 8 through 27:

B¹⁶ A "functional" fragment, derivative, mutant, or allelic variant is of sufficient length and/or structure as to possess one or more biological activities of the protein. One example of such a biological activity of the protein is formation of a coiled-coil oligomer, e.g., an octamer, for example, an octamer doughnut-shaped structure. In one embodiment, the protein derivative is conserved within the coiled-coil regions but is lacking in or mutated within one or more other regions (e.g., sequences not within the coiled-coil. Examples of suitable fragments include peptides lacking fragments which encode or stabilize the coiled coil, for example, amino acids 12-48, or the peptides depicted in Figure 11B (SEQ ID NOS:18-20). One example includes fragments which lack all or part of the region C-terminal to the proline bend (e.g. C-Terminal to Pro49). Another fragment includes the coiled coil and nuclear localization signal (e.g., amino acids 12-88); or solely the nuclear localization signal (amino acids 68-88). Xia et al., J. Virol. 66:914-21 (1992). Yet another example includes HDAG which encodes the coiled-coil region but is lacking all or a portion of the nuclear localization signal. In one embodiment, all or a portion of one or both termini of a monomer is absent or mutated. For example, the C region of the peptide (e.g., residues 50-60) can be mutated or all or a portion can be eliminated. Yet another example of derivatives includes peptides which possess amino acid modifications or additions which are characterized by a functional group which can react with a compound substituted by a "binding moiety", such as those described above or with a cross-linking agent.

At page 26, lines 5 through 22:

B17

The coding regions of the nucleic acid molecule code for HDAG and the binding moiety or moieties and any polypeptide linkers present. Where the binding moiety is a native ligand or cellular surface protein (e.g. a cellular receptor), or a binding fragment thereof, the nucleic acid molecule coding regions can correspond to the native sequences which encode a binding moiety. Because many amino acids are encoded by a plurality of codons, the coding sequence can be mutated to result in the same amino acid sequence. This may be advantageous where a codon is preferred by the selected host cell. In one embodiment, the HDAG gene can be altered such that the codons conform to the known codon use preferences for *E. coli*. See Figure 9 (SEQ ID NO:10) and Figures 15-17 (SEQ ID NOS:21, 10 and 25). The gene can be inserted into a convenient expression vector which allows production of several forms of the capsid protein including residues 1-84 (terminated in the middle domain), the short isoform and the long isoform. Dingle et al., *J. Virol.*, (1998). All three forms express well. Preferably, the nucleic acid molecule comprises the or corresponding coding nucleotide sequence of Figure 9, 10, 15-16 (SEQ ID NOS: 10, 12-14, 21, 23), or substantially the same sequences thereof, or the complement thereof. In another embodiment, the nucleic acid molecule does not possess the nucleotide sequence of GenBank Accession #M28267. The nucleic acid molecule can be, for example, isolated and/or purified or recombinant.

At page 26, line 23 through page 27, line 6:

B18

In a preferred embodiment, the nucleic acid molecule comprises the nucleotide sequence depicted in Figure 9 (SEQ ID NO: 10), nucleotides 37-150 of Figure 9 (nucleotides 37-150 of SEQ ID NO:10), nucleotides 37-186 of Figure 9 (nucleotides 37-186 of SEQ ID NO:10), Figure 10 (SEQ ID NOS:12-14), nucleotides 1421-1566 of Figure 10 (nucleotides 1421-1566 of SEQ ID NOS:12-13) or nucleotides 1457-1566 of Figure 10 (nucleotides 1457-1566 of SEQ ID NOS:12-13), Figure 15 (SEQ ID NOS:21 and 23), Figure 16 (SEQ ID NO:10); or a fragment or mutation thereof, which encodes a coiled-coil oligomer. In another preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence depicted in a row of Figure 1 (SEQ ID NOS:1-8), amino acids 12-48 of a row of Figure 1 (amino acids 12-48 of SEQ ID NOS:1-8), Figure 3C (SEQ ID NO:9), Figure 9 (SEQ ID

B18
NO:11), amino acids 12-48 of Figure 9 (amino acids 12-48 of SEQ ID NO:11), Figure 10 (SEQ ID NOS:15-17), amino acids 12-88 of Figure 10 (amino acids 12-88 of SEQ ID NOS:15-17), or δ 12-60(Y). Also encompassed in the invention are complementary strands of these sequences, DNA sequences that hybridize to these sequences and RNA sequences transcribed from these sequences. Also included are fragments or mutations thereof, which encode a coiled-coil oligomer.

At page 30, line 18 through page 31, line 10:

B19
The vectors of the present invention comprise a nucleic acid molecule which encodes HDAG (e.g. an HDAG monomer). The monomer can be a subunit of an HDAG coiled-coil oligomer, e.g. an octamer. The oligomer can comprise an HDAG polypeptide as described herein. The nucleic acid molecule thus includes any of the nucleic acid molecules described herein, for example, a native (wild type) nucleic acid, or a fragment, mutant or derivative. Especially preferred are nucleic acids encoding full-length HDAG (e.g. HDAG-S or HDAG-L) or a fragment or derivative thereof, (e.g. a functional fragment) capable of forming a coiled-coil octamer (e.g. an N-terminal coiled-coil octamer). Preferred vectors comprise a nucleic acid molecule comprising nucleotide sequence depicted in Figure 9 (SEQ ID NO:10), nucleotides 37-150 of Figure 9 (nucleotides 37-150 of SEQ ID NO:10), nucleotides 37-186 of Figure 9 (nucleotides 37-186 of SEQ ID NO:10), Figure 10 (SEQ ID NOS:12-14), nucleotides 1421-1566 of Figure 10 (nucleotides 1421-1566 of SEQ ID NOS:12-13) or nucleotides 1457-1566 of Figure 10 (nucleotides 1457-1566 of SEQ ID NOS:12-13), Figure 15 (SEQ ID NOS:21, 23) and Figure 16 (SEQ ID NO:10). Preferred vectors also comprise a nucleic acid comprising a nucleotide sequence encoding a polypeptide comprising an amino acid sequence depicted in a row of Figure 1 (SEQ ID NOS:1-8), amino acids 12-48 of a row of Figure 1 (amino acids 12-48 of SEQ ID NOS:1-8), the top row of Figure 3C (SEQ ID NO:9), Figure 9 (SEQ ID NO:11), amino acids 12-48 of Figure 9 (amino acids 12-48 of SEQ ID NO:11), Figure 10 (SEQ ID NOS:15-17), amino acids 12-88 of Figure 10 (amino acids 12-88 of SEQ ID NOS:15-17), Figure 11B (SEQ ID NOS:18-20), Figure 17 (SEQ ID NO:25) or δ 12-60(Y). Other preferred vectors comprise nucleic acids comprising sequences which are the complementary strands of the above, DNA sequences

B¹⁹ which hybridize to these sequences, RNA sequences transcribed from these sequences, and fragments and mutations thereof, which encode a coiled-coil oligomer, e.g. an octamer. Vectors can also comprise fusion molecules comprising HDAG and at least one binding moiety, as described herein.

At page 31, lines 19 through 23:

B²⁰ The vector can further comprise nucleic acid encoding a nuclear localization signal, e.g. an HDAG nuclear localization signal, for example, amino acids 68-88 of HDAG, as shown in Figure 9 (amino acids 68-88 of SEQ ID NO:11). The vector can also comprise an HDAG nucleic acid molecule comprising a sequence encoding a coiled coil and a nuclear localization signal.

At page 40, lines 13 through 17:

B²¹ Using procedures similar to those described above, HDAG molecules (e.g. fusion molecules) and vectors (e.g. cassette expression systems) comprising nucleic acid molecules, such as the vectors described herein, can be used for a variety of purposes. For example, the vector comprising all or part of a nucleic acid sequence of Figure 9 (SEQ ID NO:10) or Figure 15 (SEQ ID NO:21) (synthetic) can be used to overexpress the hepatitis delta antigen in bacteria.

At page 46, line 13 through page 47, line 6:

B²² Biophysical studies were undertaken to examine the coiled-coil domain of HDAG. Rozzelle, J.E., Jr. et al., Proc. Natl. Acad. USA, 92:382-386 (1995). As described in Example 1, a peptide was synthesized that corresponded to residues 12 to 60 of the δ 12-60(Y). This region includes the N-terminal heptad repeats. The peptide also included a C-terminal tyrosine so that the peptide could be labeled with I125 for use in a radioimmunoassay. The peptide sequence was conceptually divided into three segments based on the presence of two potential helix breakers Gly23 (G23) and Pro49 (P49); segments A (residues 12-24) (amino acids 1-13 of SEQ ID NOS:1-8), B (residues 25-49) (amino acids 14-38 of SEQ ID NOS:1-8), and C (residues 50-60)

B22
(amino acids 39-49 of SEQ ID NOS:1-8) (Figure 1). The full-length peptide $\delta 12-60(Y)$ and two shorter peptides that corresponded to regions A+B and B+C were synthesized. A number of biophysical experiments, including circular dichroism (CD), mass spectrometry, and analytical ultracentrifugation, clearly showed that the $\delta 12-60(Y)$ peptide was largely helical and formed a coiled coil Rozzelle, J.E., Jr. et al., Proc. Natl. Acad. USA, 92:382-386 (1995). The shorter peptides formed much less stable structures and were considerably less helical than $\delta 12-60(Y)$. Human polyclonal antibodies from hemophilic patients who were chronic carriers of HBV and HDV reacted with the $\delta 12-60(Y)$ peptide, in both an ELISA and in a sandwich radioimmunoassay. Rozzelle, J.E., Jr. et al., Proc. Natl. Acad. USA, 92:382-386 (1995), Wang, J. G. et al. J. Virol. 64:1108-1116. Subsequent studies indicated that monoclonal antibodies against the peptide recognized a conformational epitope only presented by the full-length peptide and not the shorter, extensively overlapping peptides, Rozzelle, J.E., Jr. et al., Proc. Natl. Acad. USA, 92:382-386 (1995).

At page 47, line 22 through page 48, line 17:

B23
When the HDAg open reading frame was originally examined, amino acids from residue 13 to 47 were identified as possibly forming a coiled coil. Glutaraldehyde cross-linking studies of full-length HDAg, as well as of the peptide, confirmed the formation of dimers, tetramers and higher-ordered structures, Wang, J. G. & Lemon, S.M., J. Virol., 67:446-454 (1993), Rozzelle, J.E., Jr. et al., Proc. Natl. Acad. USA, 92:382-386 (1995). The crystal structure of the peptide clearly shows how monomers come together to form antiparallel dimers as well as a higher-ordered octameric structure. The structure of $\delta 12-60(Y)$ also agrees well with previous circular dichroism studies of the peptide, which indicated that the two ends of the peptide (regions A and C) were important for the structural stability of the coiled coil. Rozzelle, J.E., Jr. et al., Proc. Natl. Acad. USA, 92:382-386 (1995). Shorter synthesized peptides that were missing either the A or C regions (A+B and B+C), were significantly less helical than the full-length peptide (A+B+C; 37%, 45% and 84% respectively at 37°C). The peptide structure shows that hydrophobic residues from the N terminus of one monomer (region A), not involved in the heptad repeat, interact with residues outside of the predicted coiled-coil domain near the C

B²³ terminus of the other monomer (region C) to form a hydrophobic core Trp20 (W20), Leu24 (L24), Trp50 (W50), Leu51 (L51) sandwiched between Arg13 (R13) and Arg24 (R24). This may stabilize the structure by keeping the ends of the helix from fraying. An additional stabilizing feature is a hydrogen bond between the sidechain of Glu45 (E45) and the indole nitrogen of Trp20 (W20). These hydrophobic residues, as well as the glutamic acid residue, are highly conserved in the 10 different strains of HDV identified to date (Figure 1) (SEQ ID NOS:1-8). In fact, they are more conserved than those residues in the heptad repeat making up the hydrophobic core of the long helix (Figure 1) (SEQ ID NOS:1-8).

At page 53, line 22 through page 54, line 11:

B²⁴ EXAMPLE 2: SYNTHETIC GENE FOR OPTIMIZED EXPRESSION OF HDAg-S
MATERIALS AND METHODS EXPRESSION PLASMIDS: pR5δV5 was constructed for the high-level expression of HDAg-S in Escherichia coli. The protein sequence of the American strain with the HDAg-S (GenBank accession no. M28267) was back-translated with the program BACKTRANSLATE. (This program was from the Wisconsin Package, versions 9.0, with E. coli codon frequencies obtained from [---](mailto:gopher://weeds.mgh.harvard.edu:70/Oftp%3Aweeds.mgh.harvard.edu/@/pub/codon/eco.cod.)

At Page 54, lines 12 through 18:

B25
The first PCR contained 4 pmol of each of the eight primers in a 100- μ l reaction mixture. Ten microliters of the first PCR was added to a second reaction mixture that contained an upstream primer (5'-GGGCATATGAGCCGTAGCGA) (SEQ ID NO:34) and a downstream (5'-GCGCCATGGTTTACGGAAAG) (SEQ ID NO:35) primer designed to amplify the desired full-length product. Both reactions involved a hot start at 94°C followed by 30 cycles of 1 min. at 94°C, 1 min at 57°C, and 1 min at 72°C, with a final 5-min extension at 72°C.

At page 56, lines 12 through 21:

B26
Initial studies with *E. coli* demonstrated poor expression of HDAG-S from the wild-type sequence. About 18% of the codons in the natural HDAG sequence are rarely used by *E. coli*. Attempted overexpression of codons that are rare in *E. coli* not only can inhibit expression but also can lead to misincorporation (Del Tito, B.J. et al., J. Bacterial, 177:7086-7091 (1995)). Therefore, a nucleotide sequence was designed which maintained the amino acid sequence, but increased the percentage of codons that were most favored for expression in *E. coli* from 26% to 85%. This optimized sequence Figure 9 (SEQ ID NO:10) was used to construct expression plasmid pR5 δ V5. Thus, a 40-fold increase in expression was obtained and the recombinant protein was purified to > 85% homogeneity.

At page 59, lines 17 through 24:

B27
Attempts to find a heavy atom derivative using the peptide with the wild-type sequence of the American strain of HDAG failed. Thus, a new peptide was synthesized with a cysteine replacing serine 22 (Ser22) (this residue demonstrates considerable variation in different strains of HDV, (Figure 1) (SEQ ID NOS:1-8)). The cysteine mutant and wild-type peptides crystallized isomorphously. The presence of cysteine 22 (Cys22) allowed the preparation of a platinum terpyridine derivative, facilitating the determination of the structure using SIRAS methods (Table 1). Retrospective examination of the model confirmed that the Pt was bound to the sulfur of cysteine 22 (Cys22).

At page 60, line 24 through page 61, line 18:

B²⁸
Although the majority of residues in the heptad repeat (Figure 3C) (SEQ ID NO:9) of the predicted coiled-coil region do pack as expected, Trp20 (W20) does not. Even though the C α -C β vector of Trp20 (W20) points out of the interface as would be expected for a sidechain in the a position of a heptad repeat, the sidechain of Trp20 is flipped away from the core of the coiled coil and into a hydrophobic region formed between segment A (residues 12-24) of one monomer, and segment C (50-60) of its partner within the peptide dimer. The dimer shows primarily hydrophobic interactions between residues in the A and C regions. Ile16 (I16), Leu17 (L17), Trp20 (W20), Trp50 (W50), and Leu51 (L51) are the sidechains primarily involved in this hydrophobic region, which is capped by the aliphatic portion of the sidechains of Arg13 (R13) and Arg24 (R24) (Figure 4). The primary non-hydrophobic, monomer-monomer interactions near this region involve the formation of a hydrogen bond between Trp20 (W20) and Glu45 (E45) (Figure 4). The heptad repeat is also unusual in that it contains a glycine at position 23. If the monomers were oriented in a parallel fashion, a large hole in the middle of the hydrophobic core of the dimer would result. However, since the strands are arranged antiparallel, the large sidechain of Ile41 (I41) packs into the hole formed by Gly23 (G23). The dimer is stabilized by hydrophobic interactions other than the residues in the heptad repeat. Residues from the N-termini of each monomer, Ile16 (I16), Leu17 (L17), Trp20 (W20) from one monomer and Trp50 (W50), Leu51 (L51), and Ile54 (I54) from the other, form a hydrophobic core which is protected from solvent by the aliphatic portions of Arg13 (R13) and Arg24 (R24). There is also a hydrogen bond between the sidechain of Glu45 (E45) and the indole nitrogen of Trp20 (W20) (O-N distance 2.8 Å).

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i - xiv).

In the Claims

Please amend Claims 11, 12 and 17 as follows:

- B29
11. (Amended) An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
- a) a nucleotide sequence depicted in SEQ ID NO:10, nucleotides 37 - 150 of SEQ ID NO:10, nucleotides 37 - 186 of SEQ ID NO:10, SEQ ID NOS:12-14, nucleotides 1421 - 1566 of SEQ ID NOS:12-13 nucleotides 1457 - 1566 of SEQ ID NOS:12-13, SEQ ID NO:21 and SEQ ID NO:23;
 - b) a complementary strand of the sequence of a);
 - c) DNA sequences that hybridize to the sequence of a) or b); and
 - d) RNA sequences transcribed from the sequences of a), b) or c), or a fragment or mutation thereof, which encodes a coiled-coil oligomer.
12. (Amended) An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
- a) a nucleotide sequence encoding a polypeptide comprising an amino acid sequence depicted in SEQ ID NOS:1-8, amino acids 12 - 48 of SEQ ID NO:1-8, SEQ ID NO:9, SEQ ID NO:11, amino acids 12 - 48 of SEQ ID NO:11, SEQ ID NOS:15-17, amino acids 12 - 88 of SEQ ID NOS:15-17, SEQ ID NOS:18-20 and SEQ ID NO:25;
 - b) the complementary strand of the sequence of a);
 - c) RNA sequences transcribed from the sequences of a) or b), or a fragment or mutation thereof, which encodes a coiled-coil oligomer.

- E2
B30
17. (Amended) An isolated and purified molecule comprising a polypeptide having an amino acid sequence selected from the group consisting of an amino acid sequence depicted in SEQ ID NOS:1-8, amino acids 12 - 48 of SEQ ID NOS:1-8, amino acids 12 - 60 of SEQ ID NOS:1-8, SEQ ID NO:9, SEQ ID NO:11, amino acids 12 - 48 of SEQ ID NO:11, amino acids 12 - 60 of SEQ ID NO:11, SEQ ID NOS:15-17, SEQ ID NOS:18-20 and SEQ ID NO:25, or a fragment or derivative thereof which forms a coiled-coil oligomer.